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ENERGY AND NITROGEN
IN THE FOOD OF THE WEANLING RAT

FACULTY OF AGRICULTURE
DEPARTMENT OF ANIMAL SCIENCE

by

IAN RAMSAY SIBBALD

FEBRUARY, 1957

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The undersigned hereby certify that they have read and recommend to the School of Graduate Studies for acceptance, a thesis entitled "Energy and Nitrogen in the Food of the Weanling Rat" submitted by Ian Ramsay Sibbald, B.Sc., M.Sc., in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ENERGY AND NITROGEN
IN THE FOOD OF THE WEANLING RAT

ABSTRACT

Following a series of preliminary trials, initiated in order to establish the suitability of methods and ration components, experiments were designed to measure the variance in the food intake, nitrogen retention and carcass composition, of the weanling rat, associated with the apparent digestible energy and nitrogen of the food.

The food intake of the weanling rat was largely controlled by the apparent digestible energy content of the food. The level of nitrogen in the ration, even when of a non-protein nature, exerted no significant influence upon food intake, although available energy consumption varied directly with the quality of the nitrogen source. Fibre per se appeared to decrease digestible energy consumption; however, between fibre levels there was little variation in available energy intake. Rats were able to eat to satisfy their energy requirements even when rations contained as much as 65% non-nutritive cellulose.

A curvilinear relationship was found to exist between the percentage of apparent digestible nitrogen

retained and the ratio of apparent digestible energy to apparent digestible nitrogen. The maxima of the curves varied according to the quality of the nitrogen source and appeared to occur when the level of available energy per gm. of digestible nitrogen fell within the range of 250 to 300 Calories. It is suggested that the determination of biological values should take account of the ratio of available energy to digestible nitrogen.

The influence of the apparent digestible energy: nitrogen ratio of the food on the carcass composition of rats was measured in one experiment. The lack of significant differences was attributed to the short period (two weeks) of exposure to the experimental rations.

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ENERGY AND NITROGEN
IN THE FOOD OF THE WEANLING RAT

A DISSERTATION
SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES
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FACULTY OF AGRICULTURE
DEPARTMENT OF ANIMAL SCIENCE

by
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Silence should be so respected that
the words which break it must leave
the world the better for their birth.

Saying attributed to
Gotama Buddha

ENERGY AND NITROGEN
IN THE FOOD OF THE WEANLING RAT

INTRODUCTION

The importance of energy in animal nutrition has been recognized for many years. Factors such as starch equivalent, total digestible nutrients, digestible energy, metabolizable energy, net energy and productive energy have been used with varying success in the formulation of rations for livestock. Nevertheless there is a lack of precise information regarding the influence which the available energy of a ration exerts on food intake, nitrogen retention and carcass composition.

The present investigation was undertaken to determine the extent to which the apparent digestible energy of a ration regulates the food intake and nitrogen retention of weanling rats. The value of the results of such an investigation may be indicated by two hypotheses. Firstly, if available energy largely controls food intake then it would be logical to include all the other essential components of a ration in a particular ratio to the available energy content rather than on a unit weight basis. Secondly, if the available energy intake largely controls nitrogen retention then for each nitrogen source, within a certain species at a particular stage of

development, there would be an optimum ratio between the available energy and available nitrogen of the food, when the criterion of measurement was nitrogen retention.

Carcass composition has been used as a criterion in the assessment of the suitability of animal feeds. Therefore the influence of the two variables, available energy and available nitrogen, on the moisture, fat and protein content of rat carcasses was investigated.

The work reported herein was conducted between May, 1955 and October, 1956.

REVIEW OF LITERATURE

There is a limited amount of literature available relative to the subject under investigation. In reviewing this literature only general comparisons have been made, as several different methods have been used for expressing available food energy; while many of the experimental findings are based on calculated rather than determined energy values. The following review is divided into three sections, food consumption, nitrogen retention and carcass composition.

Food Consumption

The prime regulator of food consumption appears to be the available energy content of the ration. Cowgill (1928) reported that the caloric intake of dogs remained relatively constant irrespective of the energy content of the food. Feeding rats rations diluted with cellulose, kaolin and water, Adolph (1947) found that the animals regulated their food intake according to the caloric content of the food. Hegsted and Haffenreffer (1949), also working with rats, interpreted their results as indicating that the food intake is controlled in proportion to the metabolic rate and reported that the mean daily caloric intake of rats varied as the mean body weight raised to the 0.88 power. This relationship

between energy intake and metabolic rate had previously been conceived by Kleiber (1947) who stated that the rate of intake of metabolizable energy is some multiple of the standard metabolic rate. Carpenter (1953) derived an equation to obtain the "appetite quotient" for rats. The two variables employed in this equation were the metabolic body weight and the metabolizable energy content of the food.

In a comprehensive review of some of the factors regulating energy intake and body weight, Mayer (1955) states that adaptation of energy intake to energy output appears to be the most important regulator of food intake. Kennedy (1952-'53) reports that changes in palatability have little effect upon the food consumption of the non-obese rat; the intact rat maintaining energy equilibrium while submitting to profound variations in diet, activity and environmental temperature. Strominger et al. (1953) reported that the weight of food consumed by rats varied inversely as the caloric density while Janowitz and Hollander (1955) noted that when 50 to 100% of the calculated caloric requirement of dogs was introduced into the stomach per fistula the oral caloric intake was not significantly different from the mean daily deficit in calories given by fistula.

Basing their calculations on productive energy values

Hill and Dansky (1950, 1954) and Dansky and Hill (1951) have stressed the remarkable ability of the chick to compensate for reduced dietary energy level by increasing feed consumption. Peterson et al. (1954), who based their calculations on estimated metabolizable energy values, noted that chicks ate to satisfy their energy requirements. Observing that on some rations chicks ate to satisfy their energy requirements Fisher and Weiss (1956) also reported that fibre per se stimulated a greater feed intake.

Peterson et al. (1954) noted that the level of protein in a ration limited food intake through its ability to limit growth. Hill and Dansky (1954) reported that protein level had little or no effect upon feed consumption.

Nitrogen Retention

Although as early as 1856 Hoppe reported that carbohydrate ingestion lowered the nitrogen excretion of dogs, a finding later corroborated by Lusk (1890) who performed a reverse experiment upon himself, it is only during the past decade that examination of the protein sparing action of energy has received wide attention.

Bosshardt and Barnes (1946) indicated that the protein utilization of growing rats and mice, as measured by the percentage of absorbed protein used for body

nitrogen gain, increased with caloric intake. In a comprehensive experiment concerning the interrelationships between the protein and caloric intake of rats Benditt et al. (1948) reported that there was a restriction in the utilization of a constant quantity of protein when the caloric intake fell below 1,240 Cal./M.²/day. Furthermore elevation of the caloric intake above this level did not increase the effectiveness of dietary protein for the synthesis of new tissue.

An increase in urinary nitrogen excretion associated with a decrease in caloric intake has been reported by Stevenson et al. (1946) and Calloway and Spector (1955). Meyer (1956), working with weanling rats, noted that an increase in the indigestible portion of a food increased the total faecal nitrogen excretion though no change in endogenous urinary nitrogen excretion was observed. When low levels of non-protein calories are fed, animals will break down food protein to satisfy their energy requirements (Pollack 1954). When the caloric intake is extremely low, body protein will be catabolized to produce energy (Schwimmer and McGavack 1948).

Further evidence of the protein sparing action of energy has been presented by Allison (1951) who reported that the nitrogen balance of dogs was decreased as the caloric intake was reduced. Similar findings

were reported by Rosenthal and Allison (1951). Swanson (1951) indicated the importance of non-protein calories in allowing nitrogen retention by rats. It has been reported (Pike et al. 1954) that the highest percentage of nitrogen retained by pregnant rats was associated with the highest non-protein caloric intake. Leverton et al. (1951) noted a reduction in the nitrogen excretion of young women when the caloric intake was increased.

Carcass Composition

The ratio of protein to available energy has been shown to exert a considerable influence upon fat deposition. Hill and Dansky (1954) added oat hulls to a 20% protein basal diet for chickens and observed that as the level of indigestible material increased the carcass fat content decreased. Peterson et al. (1954) reported that low protein diets resulted in chickens yielding carcasses high in fat content. When increasing levels of cellu-flour were included in the feed the fat content of the carcasses decreased but there was no significant change in the protein content.

SECTION 1

METHODS AND PROCEDURES

Introduction

The initial objective, which motivated the research reported in this thesis, was to determine the influence of apparent digestible energy on the food intake and nitrogen retention of weanling rats. Additional studies relating to the influence of the apparent digestible energy: apparent digestible nitrogen¹ ratio of the food on carcass composition were also planned. As the research progressed certain modifications and changes to these initial objectives were deemed necessary but these will be dealt with at the appropriate time.

Formulation of Experimental Rations

Fundamental to all experiments in the field of animal nutrition is the formulation of suitable rations. For the experiments planned it was necessary to have rations varying in nitrogen level and A.D.E. content. It was also imperative that the rations contain sufficient quantities of all other known nutrients as a nutritional deficiency might exert a profound influence upon the experimental results.

¹ In the cause of brevity these terms will in future be referred to as A.D.E. and A.D.N. respectively.

Nitrogen Source "A"

The nitrogen source to be used had to be of high quality in order to avoid the problems associated with amino acid deficiencies and imbalances. Consequently in deciding which proteins were to be used attention was focussed on the indispensable amino acid content of the proteins under consideration and the quantitative amino acid requirements of the weanling rat. The first choice fell to casein and lactalbumen in such proportions that each would supply an equal quantity of nitrogen. According to data presented by Block and Bolling (1951), this mixture of proteins was deficient in amino acids when compared to the quantitative amino acid requirements of the rat as presented by Rose (1938). Supplements of methionine, histidine and threonine were therefore added to the protein mixture. The composition of nitrogen source "A" is presented in table 1.

Table 1

The Composition of Nitrogen Source "A"

Casein	58.7 gm. ¹
Lactalbumen	68.8 gm. ¹
DL-methionine	0.5 gm.
L-histidine HCl	1.5 gm.
DL-threonine	1.0 gm.

¹ Contained 8 gm. nitrogen by analysis

Vitamins

The vitamin mixture, used in all rations, was based on that used by Jelinek et al. (1952). Only one major modification was made to the original mixture, namely the replacement of Wilson's Whole Liver Powder with 0.003 mg. of vitamin B₁₂ per 100 gm. of food. The composition of the vitamin mixture is presented in table 2.

Table 2

The Composition of the Vitamin Mixture^{1, 2}

Vitamin A palmitate (10,000 I.U./gm.)	60	gm.
Dry vitamin D ₂ (26,432 I.U./gm.)	4.54	gm.
Thiamine HCl	50	mg.
Riboflavin	100	mg.
Pyridoxine HCl	50	mg.
Niacin	50	mg.
Ca pantothenate	250	mg.
Folic acid	20	mg.
Biotin	2	mg.
B ₁₂	0.3	mg.
α tocopherol acetate	250	mg.
Menadione	20	mg.
P.A.B.A.	3	gm.
Inositol	10	gm.

¹ Made to 100 gm. with corn starch and included in rations at a level of 1%.

² A 50% aqueous choline chloride solution was added to the rations at the rate of 0.4 ml./100 gm. i.e. 0.2% choline chloride in the ration.

Salts

The salt mixture used was that of Jelinek et al. (1952) the composition of which is outlined in table 3. The mixture was included in all rations at a level of four percent.

Table 3

The Composition of the Salt Mixture

CaCO_3	534.0 gm.
MgCO_3	25.0 gm.
MgSO_4	16.0 gm.
NaCl	69.0 gm.
KCl	112.0 gm.
KH_2PO_4	212.0 gm.
Na_2HPO_4	800.0 gm.
FeSO_4	60.0 gm.
KI	0.08 gm.
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	16.2 gm.
$\text{Al}_2(\text{SO}_4)_3 \cdot \text{K}_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$	0.17 gm.
CuSO_4	0.90 gm.
ZnCl_2	0.52 gm.

Energy Sources

There is considerable discrepancy in the literature regarding the effects of carbohydrate and fat on nitrogen balance. Some workers claim that on isocaloric diets

fat exerts a greater nitrogen sparing effect than carbohydrate (Geiger 1951, Swanson 1951), while others hold that there is no difference (Goettsch 1951, Munro and Wikramanayake 1954, Calloway and Spector 1955). In order to avoid entering into, or being subjected to criticism arising out of, this controversy, it was decided to include Mazola oil at a constant level of 5% by weight in all purified rations; this level is high enough to satisfy the essential fatty acid requirement(s) of the rat.

As it was anticipated that the available energy content of the nitrogen source would not be very different from that of carbohydrates it was decided that increases in the level of the nitrogen source should be compensated for by a proportional reduction in carbohydrate content of the rations.

In order to vary the A.D.E. content of the foods it was decided that a diluent should be employed. For this purpose Alphacel¹, a non-nutritive cellulose, was selected.

The choice of a suitable carbohydrate presented certain difficulties. Five carbohydrate sources were compared in a series of initial experiments with the

¹ Prepared by the Nutritional Biochemicals Corporation, Cleveland, Ohio.

following results. Yellow corn dextrin¹ was incorporated in rations both in the state in which it was received and after being subjected to moistening followed by heating at 250°F. In both forms this dextrin caused severe diarrhoea to occur within 18 hours. The diarrhoea persisted throughout the seven-day experimental period and resulted in a loss in weight of the rats. Autoclaved starch was considered unsatisfactory as rats receiving rations containing this carbohydrate exhibited a slower rate of growth than did those receiving either starch or sucrose. In most respects there was little to choose between the latter two carbohydrates; however, as it was noted that there was a greater wastage of foods containing starch than of those containing sucrose it was decided to use finely granulated white sucrose as the digestible carbohydrate in all future experiments.

Experimental Procedures

All rats were placed in individual cages at the time of weaning and were fed the experimental rations to which they had been allotted for a ration acclimatization period of seven days. Following the acclimatization period the rats were maintained in individual metabolism cages for an experimental period

¹ Purchased from Fisher Scientific Company of Montreal.

of seven days. Rats were weighed at weaning and at the initiation and conclusion of the experimental period. Food consumption during the experimental period was recorded and faeces and urine were collected. Ad libitum feeding was practised throughout both the acclimatization and experimental periods.

Collection of Faeces

Faeces were collected daily, placed in small aluminium dishes, and dried in an air oven at 110°C. Prior to analysis the dried faeces were ground in a small Wiley mill using a 20-mesh screen.

Collection of Urine

Prior to receiving rats the metabolism cages were sprayed with a hot 2% boric acid solution. This was later changed to a saturated solution of boric acid in 95% ethanol. The urine was collected in 50% concentrated sulphuric acid. On termination of the experiment the cages were rinsed with hot water, the washings being added to the urine. The combined urine and washings were then filtered, the filter paper being washed several times with hot water; the filtrate was made up to 500 ml. with water prior to analysis.

Preparation of Carcasses for Analysis

Rats, whose carcass composition was to be studied, were slaughtered by placing them in a large jar containing chloroform soaked cotton wool. The dead

rats were then cut into very small pieces in one pint aluminium foil dishes and dried in an air oven at 110°C. for four hours. During this drying period the carcass material was periodically mixed to prevent it sticking to the walls of the dishes and to allow more uniform drying. The carcasses were then ground in a small Wiley mill using a 20-mesh screen, care being taken to remove all adhering material from the mill on the completion of the grinding process. The ground material was then replaced in the oven for a further four hours after which time it was bottled and stored in a refrigerator until required for analysis.

Methods of Chemical Analysis

The rations and faeces were analyzed for gross energy content using a Parr Oxygen Bomb Calorimeter¹ while all nitrogen determinations were by the method of Kjeldahl, using mercury as a catalyst. Carcass fat was determined by extraction in a Soxhlet apparatus for 20 hours with alcohol followed by 20 hours with ether (A.O.A.C. 1955). Individual analyses of urine, faeces and carcass composition were made for each rat on experiment. A.D.E. consumption was determined by subtracting total faecal energy from gross energy intake; A.D.N. consumption was determined in a similar

¹ Parr Instrument Company, Moline, Illinois; temperature changes registered by a Brown Electronik Recorder manufactured by Minneapolis-Honeywell Regulator Company, Philadelphia, Pennsylvania.

manner. Nitrogen retention was determined by subtracting gross urinary nitrogen excretion from the A.D.N. consumption.

Statistical Analyses

Analyses of variance and covariance as well as simple, partial and multiple correlations were widely used in the interpretation of experimental results. Except where otherwise indicated the methods of analysis followed those outlined by Snedecor (1946).

SECTION 2

A PRELIMINARY EXPERIMENT DIGESTIBLE ENERGY IN RELATION TO FOOD INTAKE AND NITROGEN RETENTION IN THE WEANLING RAT

Objective

This experiment was conducted to measure the magnitude of the variance likely to occur both within and between ration groups. The influence of both sex and litter on the experimental findings was also to be assessed. The results of this experiment would then enable future experiments to be designed on a sound biological and statistical basis.

Methods

Four male and four female weanling rats of the Sprague-Dawley strain were allotted to each of the two rations listed in table 4; the rations varying in Alphacel content by a level of 10%. The methods of procedure follow those outlined in section 1.

Table 4

Rations Fed During Acclimatization and
Metabolism Periods

	Ration 1	Ration 2
Nitrogen source "A", %	15.2	15.2
Sucrose, %	54.8	44.8
Alphacel, %	20.0	30.0
Mazola oil, %	5.0	5.0
Salts, %	4.0	4.0
Vitamin mixture, %	1.0	1.0
Analysis		
Gross energy, Cal/gm.	4.22	4.23
Nitrogen content, gm., %	2.07	2.08

Results and Discussion

The principal mean values obtained during the experiment are presented in table 5. The initial weight of the rats was the weight at the initiation of the seven-day experimental period.

Table 5¹

Mean Values for Data Relating to the Effect of A.D.E. on Food Consumption and Nitrogen Retention During the Seven-day Metabolism Period

Ration group	1(20% Alphacel)	2(30% Alphacel)
Number of rats	8(4 males, 4 females)	7(3 males, 4 females)
Initial weight, gm.	79	66
Final weight, gm.	116	100
Change in weight, gm.	37	33
Food consumption, gm.	81	83
Food digestibility, %	78	68
Energy digested, Cal.	273	248
Nitrogen digested, mg.	1530	1514
Urinary nitrogen, mg.	408	505
Nitrogen retained, mg.	1122	1008

¹ As one male rat on ration 2 lost considerable weight during the experiment, data relating to this animal have been omitted from this report.

Ration 1 with an Alphacel content of 20% had a gross digestibility of 78% while ration 2, containing 30% of Alphacel, had a digestibility level of 68%. That an increase of 10% Alphacel in the food reduced the digestibility by 10% would indicate the suitability of Alphacel as a diluent in work of this nature.

In order to measure the influence on food consumption of the A.D.E. content of the ration, an analysis of variance comparing food and A.D.E. consumption of the two groups of rats was performed. No difference in either food or A.D.E. consumption of the two ration groups was observed. However, a significant difference between the initial weights of the two groups of rats was noted, the difference being primarily a function of the superior growth-promoting effect of ration 1 during the seven-day acclimatization period. It was, therefore, considered advisable to adjust the two groups to a common initial weight by means of an analysis of covariance (Crampton, 1934). The results of this analysis indicated that although food consumption between the two groups of rats was significantly different there was no difference in A.D.E. consumption. Table 6 contains a summary of the analyses of variance and covariance referred to above.

The results of the analysis of covariance indicate that the food intake of weanling rats, receiving rations

containing approximately 2.07% nitrogen and varying in Alphacel content by 10% (20% and 30%), was significantly influenced by the A.D.E. content of the food; that is, it would appear that the rats ate to satisfy their energy requirement. It is anticipated that the increase in food consumption, resulting from a reduction in the A.D.E. content of the ration, could only occur within certain physiological limits; this having already been demonstrated for the chick (Peterson et al., 1954).

Table 6

Analyses of Variance and Covariance of Food Consumption and A.D.E. Consumption Between Two Rations Differing in Alphacel Content

Analyses	Source of Variation	Non-Adjusted		Adjusted for Initial Weight	
		Degrees of Freedom	Mean square	Degrees of Freedom	Mean square
Food consumed	Between rations	1	12	1	263 ¹
	Within rations	13	79.4	12	43.6
A.D.E. consumed	Between rations	1	2307	1	124
	Within rations	13	700	12	284

¹ Significant at 5% level.

It was also desired to determine the influence which the A.D.E. consumption may have had on the amount of nitrogen retained. The correlation between these factors was 0.928 for ration 1 (20% Alphacel) and 0.910 for ration 2 (30% Alphacel). The pooled correlation for both rations was 0.918. All these correlations were very large and highly significant indicating a strong association between A.D.E. consumption and retained nitrogen.

Since initial weight might have influenced both A.D.E. consumption ($r = 0.829$) and nitrogen retention ($r = 0.720$), it was necessary to remove the effects of initial weight from the correlation between A.D.E. consumption and nitrogen retention. This was accomplished by calculating the partial correlation between A.D.E. consumption and nitrogen retention independent of initial weight. The resulting r value of 0.829 was significant at the 1% level and indicated that approximately 69% of the variation in nitrogen retention was associated with A.D.E. consumption when the effects of initial weight were removed. A summary of the correlation coefficients is presented in table 7.

It has previously been pointed out that the rats on ration 2 (30% Alphacel) consumed a significantly greater quantity of food than did rats on ration 1 (20% Alphacel) when adjusted to a common initial weight by

covariance. As the nitrogen content of the two rations was essentially the same, it is suggested that for

Table 7
Correlation Coefficients Relative to Nitrogen
Retention, A.D.E. Consumption and
Initial Weight

Variables Correlated	r Values ¹
Retained nitrogen: digestible energy consumption:	
Ration 1 (20% Alphacel)	0.928
Ration 2 (30% Alphacel)	0.910
Rations 1 and 2 pooled	0.918
Retained nitrogen: initial weight	0.720
Initial weight: digestible energy consumption	0.829
Partial correlation ²	0.829

¹ All highly significant at 1% level.

² Partial correlation being the correlation between nitrogen retained and A.D.E. consumed when the influence of initial weight is statistically controlled.

each food nitrogen level there will be an optimum A.D.E. level, when nitrogen retention is the criterion of measurement. The optimum A.D.E. level will vary according to the quality and availability of the nitrogen source and with the species and stage of growth of the animal consuming the food.

Analysis of variance of the data relative to nitrogen retention, in which data for all eight rats

of ration 2 were included, indicated that there were no significant differences attributable to litter, sex or ration. Similar findings resulted from an analysis of covariance in which variations associated with initial weight were controlled. Later experiments were balanced as to sex and randomized as to litter but no further statistical analyses were conducted to measure the variance, in the nitrogen retention data, associated with these factors.

A statistical treatment known as "the power of the test" was conducted¹ on the data relative to nitrogen retention. The findings indicated that from a statistical standpoint the allotment of four rats to each ration would, >90% of the time, be just as satisfactory as eight rats. Taking into account the fact that trials of this nature require a considerable amount of time it was decided that future experiments would employ only four replicates. This procedure allowed a greater number of experiments to be completed.

Summary

It has been demonstrated that the food intake of two groups of weanling rats, whose rations contained respectively 20 and 30% of non-nutritive cellulose,

¹ Conducted by Dr. R. T. Berg, Assistant Professor of Animal Husbandry.

was significantly influenced by the A.D.E. content of the food. This would indicate that within physiological limits, as yet not determined, weanling rats eat to satisfy their energy requirements.

A.D.E. consumption has been shown to influence the nitrogen retention of the weanling rat. Approximately 69% of the variation in the nitrogen retention of the weanling rats used in this experiment was associated with A.D.E. consumption when the effects of initial weight were removed.

It is postulated that within limits there is an optimum A.D.E. level for each nitrogen level of a ration when the criterion of measurement is nitrogen retention.

Statistical treatment of data indicated that the A.D.E. per gm. nitrogen retained was not significantly influenced by litter, sex or ration differences. The power of the test indicated the feasibility of reducing the number of replicates from eight to four in future experiments.

SECTION 3

APPARENT DIGESTIBLE ENERGY AND NITROGEN IN THE FOOD OF THE WEANLING RAT Influence on Food Consumption, Nitrogen Retention and Carcass Composition

Objective

The experiment reported herein was designed to study the following relationships in the weanling rat: (a) A.D.E. and food consumption, (b) nitrogen intake and food consumption, (c) A.D.E. and nitrogen retention and (d) the A.D.E.:A.D.N. ratio of the diet on carcass composition.

Methods

Two male and two female rats were allotted to each of the 16 rations listed in table 8. In the formulation of these rations four levels of Alphacel were combined with four levels of nitrogen source "A". Additions of Alphacel and/or the nitrogen source were made at the expense of the sucrose portion of the ration.

The management of the rats and the collection, preparation and analyses of samples followed the methods outlined in section 1.

Since most of the variables studied were influenced by body weight it was necessary to remove any variation associated with this factor prior to determining the

Table 8
Rations¹ fed During Acclimatization and Metabolism Periods

Ration	Alphacel %	Nitrogen source "A" %	Sucrose %	Analysis	
				Gross energy Cal./100 gm.	Gross nitrogen mg./100 gm.
1a	10	10.2	69.8	420	1424
1b	10	15.2	64.8	425	2031
1c	10	20.3	59.7	432	2664
1d	10	25.4	54.6	439	3312
2a	20	10.2	59.8	412	1363
2b	20	15.2	54.8	420	2122
2c	20	20.3	49.7	429	2716
2d	20	25.4	44.6	435	3400
3a	30	10.2	49.8	418	1490
3b	30	15.2	44.8	435	2092
3c	30	20.3	39.7	428	2811
3d	30	25.4	34.6	458	3428
4a	40	10.2	39.8	426	1418
4b	40	15.2	34.8	426	2072
4c	40	20.3	29.7	433	2686
4d	40	25.4	24.6	448	3349

¹ Each ration contained 5% Mazola oil, 4% salts and 1% vitamin mixture.

magnitude of the various relationships under consideration. This could have been accomplished by covariance, as used in the previous section, but the simpler method of expressing the variables on a 100 gm. body weight basis was used. The body weight referred to was the average of the initial and final body weights during the experimental period.

Results

Food Consumption

The principal mean values, relative to food consumption, obtained in this experiment are shown in table 9, while a summary of the correlations obtained during statistical treatment of the data is presented in table 10.

Examination of the results indicated that as the level of Alphacel in the ration was increased (10, 20, 30 and 40%) the A.D.E. content decreased and the food consumption per 100 gm. body weight progressively increased. The increase was essentially proportional to the Alphacel level in the ration. Calculation of the A.D.E. consumption per 100 gm. body weight indicated that the increased food consumption compensated for the decreasing A.D.E. content of the food. Thus the A.D.E. consumption per 100 gm. body weight was essentially the same at each Alphacel level.

Table 9
Mean Values for Data Relating to the Effect of A.D.E.
and Nitrogen on Food Consumption

Ration	No. rats	Body wt. of rats gm.	Food ¹ consump- tion gm.	Gross N ¹ consump- tion mg.	Apparent Digestible				Apparent di- gestibility of food %
					Energy/ 100 gm. food Cal.	Energy/ consump- tion Cal.	Nitrogen/ 100 gm. food mg.	Nitrogen ¹ consump- tion mg.	
1a	4	76	84	1205	374	314	1299	1091	88
1b	4	83	84	1723	380	319	1888	1586	89
1c	4	87	85	2256	384	326	2483	2110	88
1d	4	85	88	2914	391	334	3056	2689	88
2a	4	79	100	1362	327	327	1192	1192	79
2b	4	86	93	1968	336	312	1911	1777	79
2c	4	96	98	2657	342	335	2400	2352	79
2d	4	87	92	3124	354	326	3146	2894	78
3a	4	81	106	1579	300	318	1286	1363	70
3b	4	86	110	2282	318	350	1864	2050	70
3c	4	82	104	2911	317	330	2542	2644	71
3d	4	85	105	3576	340	357	3066	3219	70
4a	4	78	120	1702	279	335	1189	1427	61
4b	4	79	116	2413	275	319	1777	2061	62
4c	4	77	124	3342	282	350	2259	2801	61
4d	4	83	112	3761	283	317	2950	3304	58

¹ Per 100 gm. body weight.

Table 10

Correlation Coefficients Relative to the Influence of
A.D.E., A.D.N. and Gross Nitrogen on Food Consumption

	Correlation	D.F.	Significance
<u>Gross correlations</u>			
r_{12}	.858	62	1%
r_{12} (within replicates)	.861	59	1%
r_{12} (within sex)	.858	61	1%
r_{13}	.119	62	--
r_{14}	.082	62	--
r_{23}	.247	62	--
r_{24}	.171	62	--
<u>Partial correlations</u>			
$r_{12.3}$	.861	61	1%
$r_{12.4}$	.859	61	1%
$r_{13.2}$	.088	61	--
$r_{14.2}$	-.127	61	--
<u>Multiple correlations</u>			
$R_{1.23}$	.863	61	1%
$R_{1.24}$	.860	61	1%

1 is the food consumption (gm.)/100 gm. body weight.

2 is the reciprocal of the A.D.E. (Cal.)/100 gm. food.

3 is the reciprocal of the A.D.N. (mg.)/100 gm. food.

4 is the reciprocal of the gross nitrogen (mg.)/100 gm. food.

This indicates that on diets varying in A.D.E. from 275 to 391 Cal. per 100 gm., weanling rats ate to satisfy their energy requirements.

The relationship between food consumption per 100 gm. body weight and the A.D.E. content of the ration is depicted graphically in figure 1. Each point represents the food consumption per 100 gm. body weight of individual rats plotted against the reciprocal of the A.D.E. per 100 gm. of food. The reciprocal of the A.D.E. content of the food was used in preference to the natural values because of the nature of the relationship involved. If a decrease in the A.D.E. content of the ration resulted in a proportionate increase in the food consumption then the relationship would be:

$$Y = \frac{K}{Z} \quad (1)$$

where, Y is the food consumption in grams
per 100 gm. body weight,
Z is the A.D.E. content of the food
(Cal. per 100 gm.)
K is a constant

If $\frac{1}{Z} = X$ the theoretical equation (1)

becomes:

$$Y = KX \quad (2)$$

therefore the relationship of food consumption to the

reciprocal of the A.D.E. content of the ration was studied.

A straight line was plotted (fig. 1) by the method of least squares. The regression equation obtained was:

$$Y = -0.7 + 33188X \quad (3)$$

$$\text{or } Y = a + bX \quad (4)$$

where $a = -0.7 \pm 7.68 \text{ gm.}$

$$b = 33188 \pm 2484$$

and $s_{y.x}$ (the standard error of estimate for deviations from regression)

$$= 7.28 \text{ gm.}$$

If a is equal to zero and $b = K$ then the theoretical equation (2) is essentially the same as the derived regression equation (3). A t-test indicated that a was not significantly different from zero, consequently equation (4) becomes:

$$Y = bX \quad (5)$$

The best estimate of K (equation 2) may be obtained from:

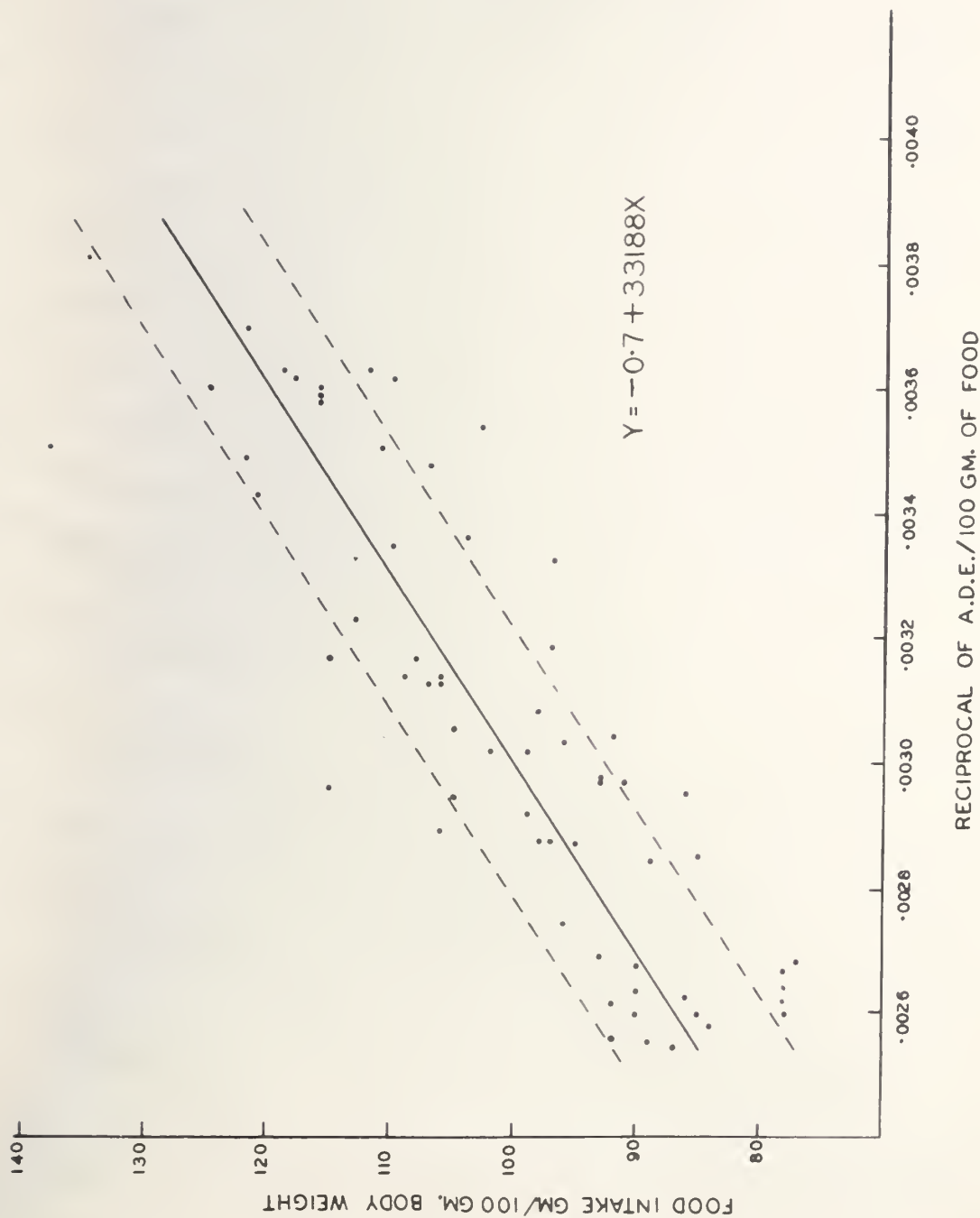
$$K = \bar{y}\bar{x} = 33064 \quad (6)$$

where, $\bar{y}$ is the mean of Y

and $\bar{x}$ is the mean of X

A t-test between b (33188 equation 4) and K (33064 equation 6) indicated no significant difference, thus the theoretical relationship shown in equation (2) is compatible with the regression equation obtained from the actual data (3).

Figure 1



Food Intake Versus the Reciprocal of the A.D.E. Content of the Food. The broken lines represent the standard error of estimate for deviations from regression

A gross correlation (r_{12}) between food consumption per 100 gm. body weight and the reciprocal of the A.D.E. per 100 gm. of food (table 10) yielded a highly significant r value of 0.858. Removing the influence of replication (r_{12} within replicates) and sex (r_{12} within sex) from the above correlation resulted in no change in the r values obtained (0.861 and 0.858) indicating that the gross correlation (r_{12}) was in no way forced by either replication or sex differences. It may therefore be seen that in this experiment approximately 74% of the variance in the food consumption of weanling rats was associated with the A.D.E. content of the food. The original standard deviation in food consumption was 14.10 gm. and the standard error of estimate for deviations from regression ($s_{y.x}$), which indicates the variation remaining after removing that associated with the A.D.E. content of the food, was 7.28 gm.

The gross nitrogen content of the rations is listed in table 8, while the nitrogen consumption, both gross and digestible, per 100 gm. body weight is recorded in table 9. These results indicate that there was no relationship between gross nitrogen or A.D.N. consumption and food or A.D.E. intake. Statistical support for these observations was obtained from a series of correlations. When the reciprocals of the A.D.N. and gross nitrogen content of the rations were correlated

with food consumption non-significant r values of 0.119 and 0.082 were obtained. The partial correlations $r_{12.3}$ and $r_{12.4}$, in which the relationship between food consumption per 100 gm. body weight and the A.D.E. content of the rations was measured, the influence of the A.D.N. and gross nitrogen content of the foods being removed, were not different from the gross correlation r_{12} (0.861, 0.859 and 0.858 respectively). These findings together with the partial correlations $r_{13.2}$ and $r_{14.2}$, measuring the relationships between food consumption and A.D.N. and gross nitrogen respectively when the influence of A.D.E. was removed, which were of negligible size (0.088 and -0.127 respectively), further emphasize that neither the gross nitrogen nor the A.D.N. content of the rations exerted any effect upon food consumption.

Peterson et al. (1954) noted that the protein content of a ration exerted an influence upon the feed consumption of chickens through its ability to limit growth. In this experiment the influence of weight changes was controlled by considering the average weight throughout the experiment. Therefore, a relationship such as that reported by Peterson et al. (1954) was not measured. It is suggested that in a short period in which change in weight, relative to total body weight is small, the nitrogen level of a ration exerts no measurable influence

on food consumption.

Nitrogen Retention

The principal mean values, relative to nitrogen retention, obtained in this experiment are shown in table 11A. The nitrogen consumption per 100 gm. body weight increased as the A.D.E.:gross nitrogen ratio of the rations decreased, since the rats ate to satisfy their energy requirements. The percentage of gross nitrogen apparently digested decreased ($P < 0.01$) as the level of Alphacel in the rations increased. This supports the work of Meyer (1956) who reported an increase in the metabolic faecal nitrogen of the rat when the cellulose content of the food was increased. Similar relationships for the pig have been reported by Crampton et al. (1955) and Lloyd and Crampton (1955).

The percentages of gross nitrogen and A.D.N. retained were not related to the nitrogen intake but were found to be largely influenced by the A.D.E. content of the rations. It was anticipated that the relationship between the percentage A.D.N. retained and the A.D.E. per gm. A.D.N. in the food would be curvilinear as on high nitrogen rations some of the nitrogen source would be used to supply energy resulting in a large urinary nitrogen excretion, while on low nitrogen diets the nitrogen intake would be limited and hence the endogenous nitrogen excretion would represent a larger percentage of the A.D.N.

Table 11

Mean Data Relating to (a) The Influence of A.D.E. on Nitrogen Retention
and (b) Carcass Composition of Experimental Animals

Ration	A					B			
	Nitrogen consumed/ 100 gm. body wt. mg.	Nitrogen digested %	Nitrogen retained %	A.D.N. retained %	A.D.E./gm. A.D.N. in food Cal.	Moisture %	Fat %	Protein %	
1a	1205	91	80	87	288	69.6	11.4	15.5	
1b	1723	92	73	78	201	70.7	10.2	16.0	
1c	2256	93	60	64	155	70.4	10.6	16.6	
1d	2914	92	47	51	128	69.6	11.9	16.4	
Mean		92				70.1	11.0	16.1	
2a	1362	87	76	87	274	69.7	11.5	15.4	
2b	1968	90	67	74	176	70.4	9.5	16.1	
2c	2657	88	58	66	143	70.9	10.5	16.8	
2d	3124	92	47	51	112	70.7	9.8	16.5	
Mean		89				70.4	10.3	16.2	
3a	1579	86	76	88	233	71.7	8.4	15.6	
3b	2282	89	59	66	170	71.5	8.4	16.3	
3c	2911	90	53	58	125	71.4	8.6	16.2	
3d	3576	89	45	50	111	71.9	8.7	16.3	
Mean		88				71.6	8.5	16.1	
4a	1702	84	68	81	235	71.7	8.9	15.4	
4b	2413	86	56	65	155	71.3	9.0	16.2	
4c	3342	84	44	52	126	70.8	10.1	15.8	
4d	3761	88	41	46	96	72.1	8.6	16.9	
Mean		86				71.7	9.2	16.1	

as the intake of the latter decreased.

Subjecting the nitrogen retention data to statistical analysis yielded the following information. The association between the percentage A.D.N. retained and the A.D.E. per gm. A.D.N. in the food yielded a highly significant r value of 0.906 at 62 D.F. A test for curvilinearity of regression demonstrated a significantly ($P < 0.01$) improved relationship; a highly significant R value of 0.932 at 61 D.F. indicates an association of 87% between the variables. The quadratic regression equation for the curvilinear relationship is:

$$Y = -4.3 + 0.6768X - 0.001223X^2$$

where Y is the percentage A.D.N. retained and X the A.D.E. per gm. A.D.N. in the food. This equation yielded a maximum value for Y of 89.3% when X equalled 276.7 Cal. A.D.E. per gm. A.D.N. The original standard deviation in the percent A.D.N. data was 14.8% and the standard error of estimate for deviations from curvilinear regression ($s_{y.x}$) 5.36% which indicates the variation remaining after removing that associated with A.D.E.:A.D.N. ratio of the food.

Carcass Composition

The mean values relating to the moisture, fat and protein content of the carcasses of the rats used in this experiment are reported in table 11B. It is of interest to note that although the rats were fed the experimental

rations for a period of two weeks (one week acclimatization, one week experimental), during which time they increased their body weights by as much as 200%, there was very little variation in carcass composition between ration groups. A slight tendency for the moisture content of the carcasses to increase with the Alphacel content of the diets was noted, while the fat content tended to decrease. The protein content of the carcasses was very uniform between Alphacel levels although within Alphacel levels the low nitrogen rations tended to result in lower protein carcasses.

Discussion

The results of this experiment have demonstrated that 74% of the variation in food consumption was associated with the A.D.E. content of the ration. The data of table 9 indicate that in seven days weanling rats eat approximately 330 Cal. A.D.E. per 100 gm. body weight. The statistical findings associated with the nitrogen retention data indicate that a level of 276.7 Cal. A.D.E. per gm. A.D.N. in the food resulted in a maximum percentage of the A.D.N. being retained. These results would suggest that in the formulation of rat rations the bulk should be so controlled that it is physiologically possible for the animals to consume 330 Cal. A.D.E. per 100 gm. average body weight per week and that the A.D.E.

per gm. A.D.N. of the food should be in the region of 277 Cal. At energy levels lower than 277 Cal. the nitrogen fraction of the diets will be inefficiently utilized while at higher energy levels no better use of the nitrogen will be made and, as the food intake is largely controlled by the A.D.E. content of the ration, the rate and efficiency of growth could be conceivably reduced due to sub-optimum nitrogen intake. At exceedingly high A.D.E. levels relative to A.D.N., nitrogen starvation of the animals is a possibility.

Working with chicks Hill and Dansky (1954) and Peterson et al. (1954) have shown that the energy:protein ratio of the feed exerts a considerable influence upon fat deposition in the carcass. As the ratio of energy to protein decreased a decrease in body fat was observed. Although the results of the present experiment demonstrated no major changes in carcass composition relative to changes in the A.D.E.:A.D.N. ratio of the food it is believed that such changes would have occurred had the animals been exposed to the experimental rations for a longer period. Unfortunately it was necessary to limit the experimental period to seven days, for the rats were growing rapidly and variations in weight, resulting from the feeding of rations differing in nutritive value, would have been magnified if a longer experimental period had been employed. Unpublished data (Bowland 1956)

indicate that variations in weight and/or age may have an important bearing upon energy intake. Due to the limited number of cages available it was impossible to continue feeding the rats on the experimental rations after termination of the experiment proper, therefore it was decided that in future, studies on carcass composition would be omitted.

Summary

It has been demonstrated that on rations varying in A.D.E. content from 275 to 391 Cal. per 100 gm., weanling rats ate to satisfy their energy requirements. The nitrogen content of the ration appeared to exert a negligible influence upon food consumption. Mean values indicate that weanling rats will consume approximately 330 Cal. per 100 gm. average body weight per week.

Nitrogen retention was largely controlled by the ratio of A.D.E. per gm. A.D.N. in the food. Statistical treatment indicated that a level of 277 Cal. A.D.E. per gm. of A.D.N. was optimal for maximum nitrogen utilization.

No differences in carcass composition resulted from maintaining weanling rats for a period of two weeks on the experimental diets. It was assumed that the short experimental period limited the manifestation of such differences.

SECTION 4

THE FOOD INTAKE AND NITROGEN RETENTION OF WEANLING RATS FED PROTEIN-FREE RATIONS

Introduction

The classical studies of W. C. Rose at Illinois have enabled the formulation of growth promoting rations devoid of protein but containing the indispensable amino acids. Rose et al. (1949) using weanling rats supplemented rations containing the 10 indispensable amino acids, in the smallest quantities compatible with maximum growth, with certain nitrogen containing compounds. It was found that all supplements used resulted in a marked acceleration in growth, diammonium citrate being the most active. Womack et al. (1953) were unable to improve negative nitrogen balances occurring in adult rats, fed low levels of the indispensable amino acids, by supplementing the diets with levels of 0.73 and 2.5% diammonium citrate. Finlayson and Baumann (1956) reported that the addition of 10% diammonium citrate to a purified rat diet resulted in a growth depression of 27% over a five week period when the rats were fed ad libitum.

In a report of an earlier experiment (section 3), in which a casein:lactalbumen mixture supplemented with amino acids was used as the nitrogen source, it was

noted that 74% of the variance in the weight of food consumed by weanling rats was associated with the reciprocal of the A.D.E. per 100 gm. of food. It was also observed that a curvilinear relationship existed between the percentage A.D.N. retained and the A.D.E. per gm. A.D.N. in the food.

Objective

The experiment reported herein was designed to study: (a) the influence of A.D.E. on the food consumption and nitrogen retention of the weanling rat when the nitrogen source of the ration consisted of a mixture of indispensable amino acids and diammonium citrate, and (b) the influence of the density and the gross nitrogen level of the rations on food consumption.

Methods

The composition of nitrogen source "B" which was used in this experiment is outlined in table 12. In the formulation of this mixture the indispensable amino acids were included in the same quantities as they were calculated to occur in nitrogen source "A", a casein:lactalbumen mixture supplemented with amino acids (section 1). Diammonium citrate and corn starch were added to the amino acid mixture in such proportions

Table 12
Composition of Nitrogen Source "B"

Nitrogen Source "B"		
	Wt. of nutrients used gm.	Wt. of active amino acids gm.
L-Arginine HCl	4.9	4.1
L-Histidine HCl	5.3	3.9
DL-Isoleucine	15.0	7.5
L-Lysine HCl	11.8	9.5
DL-Methionine	5.8	5.8 ²
DL-Phenylalanine	6.7	5.7
DL-Threonine	11.4	5.7
DL-Valine	14.2	7.1
L-Leucine	11.0	11.0
DL-Tryptophan	1.9	1.9
Diammonium Citrate	37.9	-
Corn Starch	4.6	-
Total	130.5	-
% Nitrogen	12.57	12.57

¹ Block, R. J. and D. Bolling. 1951. The amino acid composition of proteins and foods. Charles C. Thomas, Publ., Springfield, Illinois.

² Although this value is low there is sufficient tyrosine present in casein and lactalbumen to supplement the phenylalanine. Womack and Rose (1946) have shown that tyrosine can reduce the phenylalanine requirement of the rat from 0.9 to 0.45% of the total diet.

Table 13
Rations¹ fed During Acclimatization and Metabolism Periods

Ration	Alphacel %	Nitrogen source "B" ² %	Analysis			Density gm./ml.
			Sucrose %	Gross energy Cal./100 gm.	Gross nitrogen mg./100 gm.	
1a	10	10.2	69.8	409	1351	.518
1b	10	15.2	64.8	409	1903	.532
1c	10	20.3	59.7	407	2507	.460
1d	10	25.4	54.6	413	3211	.433
2a	20	10.2	59.8	409	1330	.402
2b	20	15.2	54.8	405	1955	.417
2c	20	20.3	49.7	415	2583	.398
2d	20	25.4	44.6	419	3112	.353
3a	30	10.2	49.8	410	1350	.333
3b	30	15.2	44.8	413	1923	.328
3c	30	20.3	39.7	422	2522	.338
3d	30	25.4	34.6	424	3211	.307
4a	40	10.2	39.8	408	1338	.287
4b	40	15.2	34.8	418	1973	.277
4c	40	20.3	29.7	420	2529	.268
4d	40	25.4	24.6	426	3168	.253

¹ Each ration contained 5% Mazola oil, 4% salts and 1% vitamin mixture.

² Nitrogen Source "B" composition listed in table 12.

that the nitrogen content of source "B" was the same as that of nitrogen source "A".

In the formulation of the experimental rations four levels of Alphacel were combined with four levels of nitrogen; additions of Alphacel and/or the nitrogen source being made at the expense of the sucrose portion of the ration. This design is identical with that used in an earlier experiment (section 3). The composition of the experimental rations is listed in table 13.

As it was anticipated that there would be a greater within ration variation exhibited by rats consuming nitrogen source "B" than there was when rats were fed nitrogen source "A" (sections 2 and 3) it was decided that three male and three female rats should be allotted to each of the 16 rations listed in table 13. The methods and procedures used in obtaining the experimental results were as outlined in section 1.

As in the previous section data were collected separately for individual rats and all subsequent computations and statistical analyses are based on individual and not group average data. Since most of the variables studied were influenced by body weight, it was necessary to remove any variation associated with this factor prior to determining the magnitude of the various relationships under consideration. This was accomplished by expressing the variables on a 100 gm.

body weight basis. The body weight referred to was the average of the initial and final body weights of the rats during the experimental period.

Results

Food Consumption

The principal mean values, relative to food consumption, are shown in table 14. Examination of these results indicates that as the level of Alphacel in the ration was increased (10, 20, 30 and 40%) the A.D.E. content decreased and the food consumption per 100 gm. body weight increased. The A.D.E. consumption per 100 gm. body weight remained relatively constant between ration groups indicating that the decreasing A.D.E. levels of the rations were compensated for by increased food consumption. The increase in food consumption resulted in an increase in gross nitrogen intake.

An analysis of variance indicated a highly significant ($P < 0.01$) difference in the weight of food consumed per 100 gm. body weight between Alphacel levels. No such difference was found when the food consumption between nitrogen levels was compared. The interaction between Alphacel and nitrogen levels was not significant. These results indicate that food consumption was neither stimulated nor depressed by increasing the level of the

Table 14
Mean Values for Data Relating to the Effect of A.D.E.
and Gross Nitrogen on Food Consumption

Ration	Body wt. of rats gm.	Food consumption/ 100 gm. body wt. gm.	Food consumption/ 100 gm. body wt. ml.	Gross nitrogen consumption/ 100 gm. body wt. mg.	A.D.E.			
					/100 gm. food Cal.	/100 ml. food Cal.	/100 gm. body wt. Cal.	gm. wt.
1a	50	68	131	919	368	191	250	
1b	52	79	148	1503	368	197	291	
1c	58	76	165	1905	365	168	278	
1d	53	76	176	2440	373	161	283	
2a	48	79	197	1051	333	134	264	
2b	56	83	199	1623	328	138	274	
2c	61	78	196	2015	334	133	260	
2d	54	82	232	2552	342	122	282	
3a	51	85	255	1148	296	98	251	
3b	54	90	274	1731	296	96	266	
3c	54	87	258	2194	297	100	257	
3d	50	89	290	2858	308	94	273	
4a	51	100	348	1325	257	75	258	
4b	53	107	386	2111	270	75	289	
4c	54	106	395	2681	266	71	281	
4d	47	98	387	3105	266	70	272	

protein-free, diammonium citrate containing, nitrogen source "B". A further test of the influence of the gross nitrogen level of the ration on food consumption was obtained from a correlation between the food consumption (gm.) per 100 gm. body weight and the reciprocal of the gross nitrogen per 100 gm. of food. The r value of -0.091 (94 D.F.) was not significant indicating that changing levels of nitrogen source "B", and therefore diammonium citrate, did not influence food consumption. This indicates that diammonium citrate did not depress food intake unless it had already expressed itself to a maximum at the lowest level at which it was included in the experimental rations.

The statistical analyses relative to the influence of A.D.E. and ration density yielded some interesting findings. A correlation between the weight of food consumed per 100 gm. body weight and the reciprocal of the A.D.E. per 100 gm. of food gave a highly significant r value of 0.763 at 94 D.F. A similar correlation between the volume of food consumed and the reciprocal of the A.D.E. per 10 ml. of food yielded the significantly higher ($P < 0.01$) r value of 0.964 at 94 D.F. This difference in r values could not be explained by a change in the volume of food consumed being associated with a change in the reciprocal of the A.D.E. consumed per 100 gm. body weight for a correlation between these

two variables yielded an r value of -0.015 at 94 D.F., which did not even approach a level of significance. This was further emphasized by an analysis of variance which found no difference in the A.D.E. consumption of the rats between varying food density levels.

Density did have a profound influence upon both the weight and volume of food consumed but this could be explained by the very high association between the reciprocal of the A.D.E. content of the food and the density. A correlation between the reciprocal of the A.D.E. per 100 gm. of food and the density yielded an r value of -0.883 at 94 D.F. while a similar correlation between the reciprocal of the A.D.E. per 100 ml. of food and the density gave an r value of -0.946 at 94 D.F. These latter two r values (-0.883 and -0.946) were significantly ($P < 0.001$) different. This indicates that changes in the reciprocal of the A.D.E. per 100 ml. of food were more closely associated with changes in density than were changes in the reciprocal of the A.D.E. per 100 gm. of food. This explains why the association between the volume of food consumed per 100 gm. body weight and the reciprocal of the A.D.E. per 100 ml. of food is significantly ($P < 0.01$) greater than that between the weight of food consumed and the reciprocal of the A.D.E. per 100 gm. of food.

Nitrogen Retention

The principal mean values relative to nitrogen retention, obtained in this experiment, are shown in table 15. Increasing levels of Alphacel tended to decrease the digestibility of the gross nitrogen of the food though no difference was observed between Alphacel levels of 30 and 40 percent. The consumption of A.D.N. per 100 gm. body weight increased as the A.D.E. per gm. A.D.N. of the food decreased since variance in food consumption was largely associated with the A.D.E. content of the ration. The percentage A.D.N. retained did not appear to be influenced by the nitrogen (and therefore diammonium citrate) intake, but rather by the A.D.E.:A.D.N. ratio of the food.

Statistical analysis yielded the following information. A correlation between the percentage A.D.N. retained and the A.D.E. per gm. A.D.N. in the food yielded a highly significant r value of 0.739 at 94 D.F. A test for curvilinearity of regression demonstrated a significantly ($P < 0.01$) improved relationship, a highly significant R value of 0.807 at 93 D.F. indicating an association of 65% between the variables. The quadratic equation obtained for the curvilinear regression was:

$$Y = -2.8 + 0.507X - 0.000959X^2$$

where Y is the percentage A.D.N. retained and X the A.D.E.

Table 15
Mean Data Relating to the Influence of A.D.E. on Nitrogen Retention

Ration	Gross Nitrogen		A.D.N.		Retained %	A.D.E./gm. A.D.N. in food Cal.
	/100 gm. Food mg.	Digested %	/100 gm. Food mg.	/100 gm. body wt. mg.		
1a	1351	92	1229	836	58	298
1b	1903	92	1745	1378	59	211
1c	2507	91	2277	1730	51	161
1d	3211	92	2974	2260	51	125
Mean		<u>92</u>				
2a	1330	85	1125	889	66	298
2b	1955	87	1699	1410	61	193
2c	2583	89	2298	1792	54	146
2d	3112	92	2858	2344	<u>44</u>	120
Mean		<u>88</u>				
3a	1350	81	1094	930	68	272
3b	1923	80	1531	1378	65	196
3c	2522	85	2143	1864	46	139
3d	3211	90	2892	2574	42	106
Mean		<u>84</u>				
4a	1338	81	1078	1078	60	239
4b	1973	84	1663	1779	52	163
4c	2529	83	2096	2222	48	128
4d	3168	86	2716	2662	34	98
Mean		<u>84</u>				

per gm. A.D.N. in the food. This equation yielded a maximum for Y of 65.2% when X equalled 264 Cal. A.D.E. per gm. A.D.N. The original standard deviation in the percent A.D.N. retained data was 11.1% and the standard error of estimate for deviations from curvilinear regression ($s_{y.x}$), which indicates the variation remaining after removing that associated with the A.D.E.:A.D.N. ratio of the food, was 6.6 percent.

Discussion

Food Consumption

The results of this experiment indicate that the nitrogen level of the ration, and hence the diammonium citrate content, did not exert any significant influence upon food intake. It was noted that 58% of the variance in the weight of food consumed was associated with the reciprocal of the A.D.E. per 100 gm. of food while a significantly ($P < 0.01$) greater association of 93% was found to exist between the variance in the volume of food consumed and the reciprocal of the A.D.E. per 100 ml. of food. Density was found to have no influence upon the A.D.E. consumption of the rats though it did appear to influence food intake. An explanation of the latter point is that the A.D.E. content and the density of the food were very closely associated. The

correlation between the reciprocal of the A.D.E. per 100 ml. of food and the density ($r = -0.946$ at 94 D.F.) was significantly greater ($P < 0.01$) than that between the reciprocal of the A.D.E. per 100 gm. of food and the density ($r = -0.883$ at 94 D.F.). This indicates that a slight change in the reciprocal of the A.D.E. content of the food is more greatly reflected in a change in volume than a change in weight. This probably explains why the association between the volume of food consumed per 100 gm. body weight and the reciprocal of the A.D.E. per 100 ml. of food (93%) is significantly greater than that between the weight of food consumed and the reciprocal of the A.D.E. per 100 gm. of food.

Nitrogen Retention

The statistical analysis of the nitrogen retention data indicates a curvilinear relationship between the percentage A.D.N. retained and the A.D.E. per gm. A.D.N. A maximum of 65.2% of the A.D.N. was retained when the A.D.E. per gm. A.D.N. was 264 Calories. Using a nitrogen source based on a casein:lactalbumen mixture supplemented with amino acids, (section 3) a similar maximum of 89.3% when the A.D.E. per gm. A.D.N. was 277 Cal. was derived. The similarity of the two optimal levels of A.D.E. per gm. A.D.N. (264 and 277 Cal.) raises the question as to whether the optimal ratio of A.D.E. per gm. A.D.N. for all nitrogen sources

falls in close proximity to these values.

Several interesting points arise from the nitrogen retention data of this experiment and that reported in section 3. In the determination of the biological values of proteins it is generally considered that low levels of protein yield the most accurate results. It is widely accepted that as the level of protein in the ration increases the biological value decreases (Barnes et al. 1946). However, if, as has been shown, the relationship between nitrogen retention and the A.D.E. per gm. A.D.N. in the food is curvilinear, then it would appear that it is not so much the level of protein in the food which is important but rather the ratio of A.D.E. to A.D.N.

The percentage A.D.N. retained corresponds to the biological value for growth and differs from the biological values obtained by the Thomas-Mitchell method in as much as the latter makes correction for the metabolic faecal and the endogenous urinary nitrogen excretion. Biological values are frequently expressed as percentages of the biological value of a protein known to be of high quality. A comparison of this nature in which the percentage A.D.N. retained by rats consuming nitrogen source "B" is expressed as a percentage of the percentage A.D.N. retained by rats

consuming nitrogen source "A" (section 3) at varying levels of A.D.E. per gm. A.D.N. in the food, is presented in table 16.

Table 16

The Influence of the A.D.E.:A.D.N. Ratio on the Biological Value for Growth¹

	A.D.E. per gm. A.D.N. Cal.	Nitrogen Source "A"						
		100	150	200	250	264	277 ²	300
Nitrogen Source "B"	100	<u>74.9</u>	55.0	46.6	43.3	43.0	42.9	43.2
	150	101.0	<u>74.1</u>	62.9	58.4	58.0	57.8	58.3
	200	117.8	86.4	<u>73.3</u>	68.1	67.6	67.4	67.9
	250	125.1	91.8	77.9	<u>72.4</u>	71.8	71.6	72.2
	264 ²	125.5	92.1	78.2	72.6	<u>72.0</u>	71.9	72.4
	277	125.2	91.9	78.0	72.4	71.9	<u>71.7</u>	72.2
	300	122.2	90.4	76.7	71.2	70.7	70.5	<u>71.0</u>

¹ The percentage A.D.N. retained of Nitrogen Source "B" expressed as a percentage of the percentage A.D.N. retained of Nitrogen Source "A" at varying ratios of A.D.E.:A.D.N. Data relative to Nitrogen Source "A" from section 2.

² Level of A.D.E. per gm. A.D.N. which results in a maximum percentage of A.D.N. being retained.

The data of table 16 indicate that at equal levels of A.D.E. per gm. A.D.N. the biological value for growth of nitrogen source "B", expressed as a percentage of that for nitrogen source "A" (figures underlined), is not constant but changes with the A.D.E.:A.D.N. ratio of the

food. When the level of A.D.E. per gm. A.D.N. for the two nitrogen sources differs, the percentage biological value for growth of nitrogen source "B" exhibits even greater variation except in the range 250 to 300 Cal. per gm. A.D.N. which occupies the plateaux of the two curves. It would therefore appear logical to determine the biological values of proteins, or nitrogen sources, either at some constant level of A.D.E. per gm. A.D.N. which ensures that the percentage A.D.N. retained is close to a maximum (i.e. on the plateau of the curve) or at a level of A.D.E. per gm. A.D.N. which results in a maximum percentage of A.D.N. being retained. Table 16 demonstrates quite clearly that as one moves away from the plateaux of the curves greater variation is encountered.

Summary

Variations in the food consumption of weanling rats, fed rations containing a mixture of the indispensable amino acids and diammonium citrate, were largely associated with the A.D.E. content of the rations. As the A.D.E. level of a ration was closely associated with its density the best measure of the influence of A.D.E. on food intake was obtained by a correlation between the volume of food consumed and the reciprocal of the A.D.E. per 100 ml. of food (93% association). The gross nitrogen

level of the rations, and hence the diammonium citrate content, did not exert any significant influence on food consumption.

The ratio of A.D.E. to A.D.N. was associated with 65% of the variance in the percentage A.D.N. retained. This relationship was curvilinear yielding a maximum for the A.D.N. retained of 65.2% when the A.D.E. per gm. A.D.N. was 264 Calories.

The importance of A.D.E. in determining the biological values of proteins or nitrogen sources is indicated. It would seem necessary to determine biological values when the ratio A.D.E.:A.D.N. of the food results in the percentage of A.D.N. retained being situated on the plateau of the curve. Perhaps the most accurate data would be obtained when the A.D.E. per gm. A.D.N. results in a maximum percentage of A.D.N. being retained.

SECTION 5

THE INFLUENCE OF THE NITROGEN SOURCE ON THE FOOD INTAKE AND NITROGEN RETENTION OF WEANLING RATS

Objective

Earlier experiments have shown that weanling rats eat to satisfy their energy requirements and that the percentage of the A.D.N. which is retained varies with the ratio of A.D.E. to A.D.N. The experiment reported herein was designed to study: (a) the physiological maximum of food intake, (b) the influence of the nitrogen source on A.D.E. intake and (c) the relationship between the A.D.E.:A.D.N. ratio of the food and the percentage A.D.N. retained for six different nitrogen sources, using the weanling rat as the experimental animal.

Methods

Two male and two female weanling rats were allotted to each of 37 experimental rations. The composition of these rations is presented in tables 17 and 18. In the formulation of the rations six different nitrogen sources were used; these were: nitrogen source "A" (section 1), fishmeal, meat scrap, solvent extracted soyabean oil meal, casein and corn gluten. Variations in the levels of Alphacel and/or nitrogen sources were

Table 17

Composition of Rations Containing Nitrogen Source "A" With Additional Data Relating to Their Consumption by Weanling Rats

Ration Composition ¹				Food Analysis							
Ration	Alphacel %	Nitrogen Source "A" %	Sucrose %	Density gm./ml.	Gross Nitrogen mg./100 gm.	Gross Energy Cal./100 gm.	A.D.E./100 gm. Food Cal.	A.D.E./100 ml. Food Cal.	Food Consump- tion/100 gm. Body Wt. gm. ml.	A.D.E./ 100 gm. Body Wt. Cal.	Wt. of Rats gm.
1	30	20.3	39.7	.357	2708	423	298	106	98	292	85
2	40	20.3	29.7	.307	2791	424	260	80	111	290	82
3	45	20.3	24.7	.280	2732	427	245	69	122	298	84
4	50	20.3	19.7	.273	2760	426	227	62	132	301	82
5	55	20.3	14.7	.257	2719	432	204	52	155	315	68
6	60	20.3	9.7	.243	2680	430	186	45	161	299	65
7	65	20.3	4.7	.232	2612	426	167	39	179	299	55
8	0	10.2	79.8	.662	1304	413	401	265	86	345	54
9	10	10.2	69.8	.562	1336	410	361	202	84	303	68
10	20	10.2	59.8	.420	1362	414	330	138	94	310	73
11	30	10.2	49.8	.356	1328	412	292	104	102	298	79
12	10	11.4	68.6	.529	1488	416	365	193	87	318	71
13	20	11.4	58.6	.429	1546	416	332	142	88	292	78
14	30	11.4	48.6	.361	1512	416	297	107	102	303	85
15	20	12.7	57.3	.410	1650	422	340	140	92	313	81
16	30	12.7	47.3	.345	1702	419	300	103	99	297	90
17	20	14.0	56.0	.422	1798	419	334	140	88	294	77
18	30	14.0	46.0	.341	1835	424	306	104	102	312	74
19	10	16.5	63.5	.524	2156	421	374	194	80	299	85
20	20	16.5	53.5	.417	2232	422	340	141	90	306	84
21	10	19.0	61.0	.513	2401	427	380	194	82	312	78
22	30	19.0	41.0	.344	2544	428	301	103	99	298	88

¹All rations contained 4% salts (Jelinek et al. '52), 5% Mazola oil and 1% vitamin mix (Section 1).

Table 18

Composition of Rations Containing Nitrogen Sources Other Than "A" With Additional Data Relating to Their Consumption by Weanling Rats

Ration Composition ¹		Food Analysis					Food Consumption/100 gm. Body Wt.				A.D.E./100 gm. Body Wt.		Wt. of Rats gm.
Ration ²	Alphacel %	Nitrogen Source %	Sucrose %	Density gm./ml.	Gross Nitrogen mg./100 gm.	Gross Energy Cal./100 gm.	A.D.E./100 gm. Food Cal.	A.D.E./100 ml. Food Cal.	gm.	Wt. gm.	Cal.	gm.	
F1	10	10.0	75.0	.701	1272	386	336	235	77	110	259	52	52
F2	23	10.0	62.0	.588	1243	384	290	169	91	156	264	56	56
F3	37	10.0	48.0	.470	1232	383	234	110	117	249	274	64	64
M1	12	15.0	68.0	.687	1015	371	306	210	63	92	193	36	36
M2	25	15.0	55.0	.562	1254	374	274	154	90	160	247	41	41
M3	38	15.0	42.0	.454	1262	371	210	95	83	183	174	44	44
S1	13	15.0	67.0	.687	1176	382	321	221	86	125	276	54	54
S2	26	15.0	54.0	.576	1201	384	273	156	98	172	268	58	58
S3	39	15.0	41.0	.440	1241	384	226	100	119	270	269	62	62
C1	22	7.5	60.5	.420	1144	415	327	138	80	190	262	52	52
C2	36	7.5	46.5	.340	1183	415	278	94	92	271	256	62	62
C3	48	7.5	34.5	.292	1456	414	225	66	130	443	292	75	75
G1	23	8.0	59.0	.407	1156	413	315	129	75	183	236	44	44
G2	36	8.0	46.0	.313	1210	417	276	86	86	274	237	49	49
G3	49	8.0	33.0	.275	1171	414	217	60	106	386	230	47	47

¹All rations contained 4% salts and 1% vitamin mix. Rations prefixed C and G also contained 5% Mazola oil.

²Prefixes refer to nature of nitrogen source; F white fishmeal, M meat scrap, S solvent extracted soyabean oil meal, C casein and G corn gluten.

made at the expense of the sucrose portion of the ration. All rations contained vitamin and mineral supplements. The methods and procedures used were as outlined in section 1.

Results

Food Consumption

The principal mean values relative to food consumption, obtained in these experiments, are recorded in tables 17 and 18. It will be noted that an increase in the Alphacel content of a ration was associated with a decrease in both the density and the A.D.E. content of the food. Within any group of rations, based on a single nitrogen source, the A.D.E. intake per 100 gm. body weight was remarkably uniform; an exception to this was noted for rats consuming rations containing meat scrap. It would appear, therefore, that a decrease in the A.D.E. content of a ration was compensated for by an increase in food consumption.

The mean A.D.E. consumption per 100 gm. body weight for each of the six nitrogen sources was as follows: nitrogen source "A" 304 Cal., fishmeal 266 Cal., soyabean oil meal 271 Cal., casein 270 Cal., corn gluten 234 Cal. and meat scrap 205 Cal. Analysis of variance indicated a highly significant ($P < 0.01$) difference in the A.D.E. intake of rats between nitrogen sources with

an L.S.D. of 29 Cal. at the 1% level of significance. In the aforementioned analysis of variance and in the calculation of the L.S.D., data from only three of the 22 rations containing nitrogen source "A" were employed; this allowed for a balanced design with equal numbers from each of the six nitrogen sources. The rations represented were numbers 16, 18 and 19 (table 17) which were drawn at random.

A summary of the correlations between food consumption and the reciprocal of the A.D.E. content of the food is presented in table 19. It will be noted that correlations between the volume of food consumed and the reciprocal of the A.D.E. per 100 ml. of food yielded higher r values than those obtained when the weight of food consumed was correlated with the reciprocal of the A.D.E. per 100 gm. of food. This apparently was not the direct result of a change in food density for when rations containing nitrogen source "A" were grouped according to density no difference in the A.D.E. consumption per 100 gm. body weight was detected by analysis of variance.

The correlation between the volume of food consumed per 100 gm. body weight and the reciprocal of the A.D.E. per 100 ml. of food, for rats receiving rations containing nitrogen source "A", yielded an r value of 0.992 at 86 D.F. This indicates an almost perfect

Table 19

A Summary of Correlations Between Food Intake and the A.D.E. Content of the Food

	D.F.	r_{yx}^1	Sig.	$r_{y_1x_1}^2$	Sig.
Nitrogen Source "A"	86	0.987	1%	0.992	1%
Fishmeal	10	0.967	1%	0.985	1%
Meat scrap	10	0.416	--	0.830	1%
Soyabean oil meal	10	0.832	1%	0.971	1%
Casein	10	0.905	1%	0.964	1%
Corn gluten	10	0.882	1%	0.962	1%

$^1r_{yx}$ The correlation between the weight of food consumed per 100 gm. body weight and the reciprocal of the A.D.E. per 100 gm. of food.

$^2r_{y_1x_1}$ The correlation between the volume of food consumed per 100 gm. body weight and the reciprocal of the A.D.E. per 100 ml. of food.

straight line relationship between the two variables.

Therefore, it may be assumed that even on rations containing as much as 65% Alphacel, with as few as 39 Cal. A.D.E. per 100 ml. of food, rats were able to eat to satisfy their energy requirements.

Nitrogen Retention

Mean data relative to nitrogen retention are presented in tables 20 and 21. The data of table 20 indicate that as the indigestible portion of the ration

Table 20

Mean Data Relating to the Nitrogen Retention of Rats
Receiving Rations Containing Nitrogen Source "A"1

Ration	Nitrogen consumed /100 gm. body wt. mg.	Nitrogen digested %	A.D.N. retained %	A.D.E./gm. A.D.N. in food Cal.	Food dige- stibility %
1	2691	89	57	124	68
2	1271	87	53	107	58
3	3518	86	49	104	53
4	3655	86	48	96	49
5	4264	84	40	90	44
6	4482	82	35	84	37
7	5482	78	31	82	32
8	1125	97	78	318	98
9	1124	91	85	296	88
10	1282	88	90	276	79
11	1354	88	86	251	70
12	1302	92	88	268	88
13	1360	91	88	236	79
14	1536	88	85	222	70
15	1513	90	83	228	79
16	1669	89	79	198	70
17	1590	90	79	206	79
18	1887	88	72	188	70
19	1736	93	78	187	88
20	2007	91	73	168	79
21	1976	92	70	172	88
22	2534	88	62	134	68

1 The composition of these rations is presented in table 17.

increased the apparent digestibility of the food nitrogen decreased. A similar trend was exhibited by the data relative to rations containing fishmeal and soyabean oil meal listed in table 21.

A summary of the correlations between the percentage

Table 21

Mean Data Relating to the Nitrogen Retention of Rats
Receiving Rations Containing Nitrogen Sources
Other Than "A"¹

Ration	Nitrogen consumed /100 gm. body wt. mg.	Nitrogen digested %	A.D.N. retained %	A.D.E./gm. A.D.N. in food Cal.	Food dige- stibility %
F1	988	80	65	334	86
F2	1142	78	74	300	76
F3	1435	75	73	252	62
M1	638	63	-32	482	80
M2	1120	68	27	323	70
M3	1050	60	0	277	54
S1	1015	78	62	347	84
S2	1170	76	62	300	71
S3	1476	71	66	256	60
C1	906	85	66	337	77
C2	1086	79	70	298	65
C3	1882	86	76	180	52
G1	866	78	25	348	75
G2	1041	80	25	286	63
G3	1225	74	26	251	49

¹ The composition of these rations is presented in table 18.

A.D.N. retained and the A.D.E. per gm. A.D.N. for the six nitrogen sources is presented in table 22. The association between the percentage A.D.N. retained and the A.D.E. per gm. A.D.N. in the food, for rats fed rations containing nitrogen source "A", yielded a highly significant r value of 0.894 at 86 D.F. A test for curvilinearity of regression demonstrated a significantly

($P < 0.01$) improved relationship; a highly significant R value of 0.955 at 85 D.F. indicates an association of 91% between the variables. The quadratic regression equation for the curvilinear relationship is:

$$Y = -18.9 + 0.7946X - 0.001500 x^2$$

where Y is the percentage A.D.N. retained and X the A.D.E. per gm. A.D.N. in the food. This equation yielded a maximum value for Y of 86.3% when X equalled 265 Cal. A.D.E. per gm. A.D.N. The original standard deviation for the percent A.D.N. retained data was 18.5% and the standard error of estimate for deviations from curvilinear regression ($s_{y.x}$), which indicates the variation remaining after removing that associated with the A.D.E.:A.D.N. ratio of the food, was 5.5 percent.

Table 22

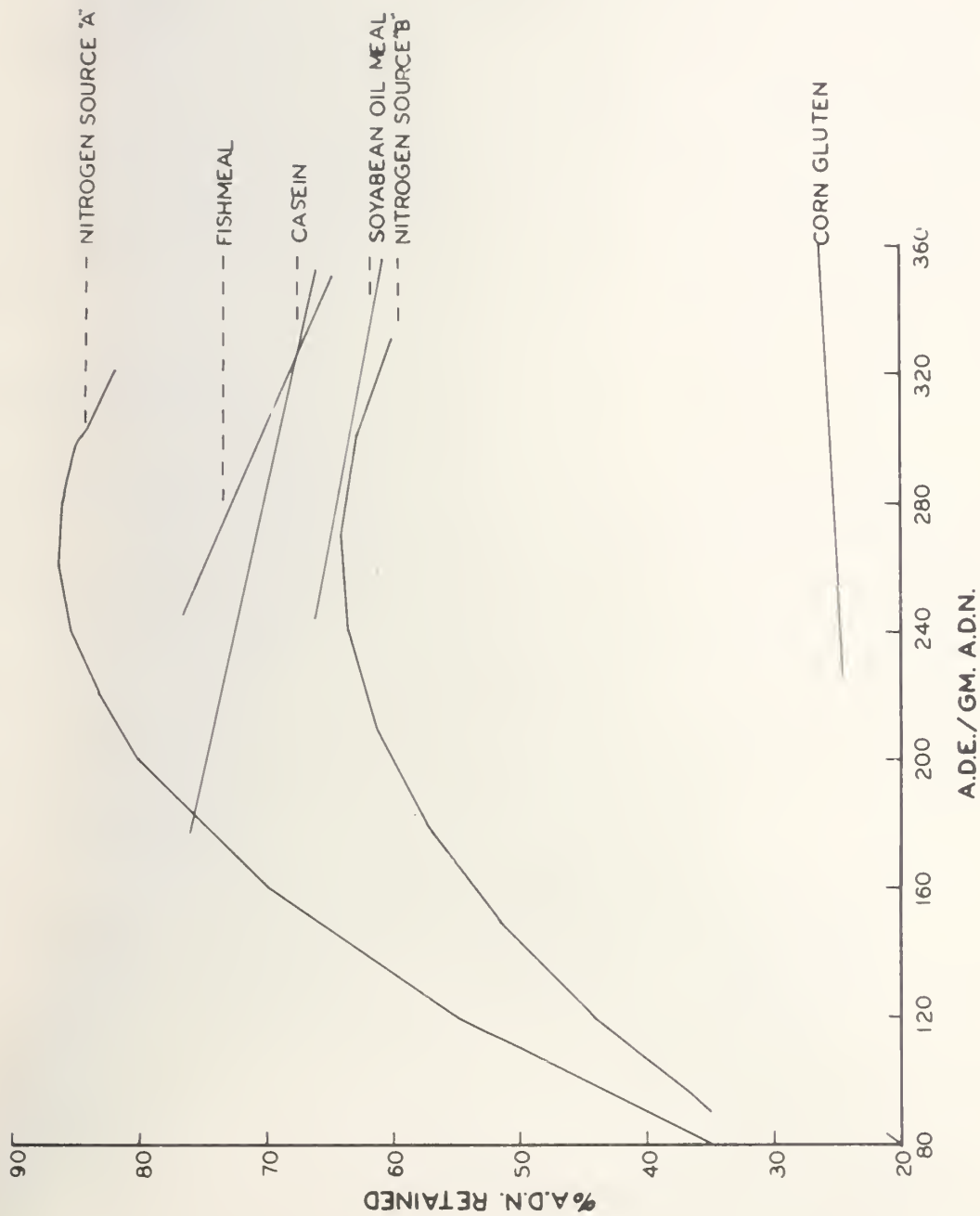
A Summary of Correlations Between the
Percentage A.D.N. Retained and the
A.D.E. per gm. A.D.N.

Nitrogen Source	D.F.	Correlation	Sig.
"A"	86	0.894	1%
"A"	85	0.955 ¹	1%
Fishmeal	10	-0.336	--
Meat scrap	10	-0.929	1%
Soyabean oil meal	10	-0.300	--
Casein	10	-0.583	5%
Corn gluten	10	0.142	--

¹ This is the multiple correlation value and takes account of the curvilinear association between the variables.

The limited number of rations employed prevented the derivation of similar quadratic equations for the other five nitrogen sources. The data of table 21 show very little variation in the percentage A.D.N. retained relative to the changes in the levels of A.D.E. per gm. A.D.N. in the food. The low r values resulting from correlations between the A.D.E. per gm. A.D.N. and the percentage A.D.N. retained for the different nitrogen sources together with the almost horizontal regression lines of fig. 2 indicate that the levels of A.D.E. per gm. A.D.N. employed resulted in percentages of A.D.N. retained which were close to the maxima, that is within the bounds of the plateaux of the curves. An exception to this was meat scrap, but this may be explained by the extremely high level of A.D.E. per gm. A.D.N. (482 Cal.) which occurred in ration M1. This high level of A.D.E. resulted in a percentage A.D.N. retained which was considerably displaced to the right of the maximum of the curve and allowed a significant negative correlation ($r = -0.929$ at 10 D.F.) to occur. As the data relating to meat scrap were atypical, the regression line relative to this nitrogen source has been omitted from fig. 2. Although the data are not conclusive it would appear that the maximum percentage of A.D.N. retained probably occurred when the A.D.E. per gm. A.D.N. fell between

Figure 2



The Influence of A.D.E. on A.D.N. Retention
(curve for nitrogen source "B" from
section 4)

250 and 300 Cal. This is supported by the positions of the maxima of the two curvilinear regression lines included in fig. 2 where more data were available.

An interesting feature of fig. 2 is the relative values of the approximate maximum percentages of A.D.N. retained. If these maxima indicate the quality of the nitrogen sources then it will be noted that nitrogen source "A" was superior to fishmeal, casein, soyabean oil meal and nitrogen source "B" which in turn were superior to corn gluten. The data relative to nitrogen source "B" are derived from an earlier experiment (section 4).

Discussion

Food Consumption

The A.D.E. intake of the weanling rats used in these experiments appeared to be influenced by the nature of the nitrogen source. Below, the nitrogen sources are listed in the approximate order of quality and compared with the average A.D.E. intake per 100 gm. body weight of the rats.

Nitrogen source "A"	304 Cal
Fishmeal	266 Cal ²
Soyabean oil meal	271 Cal ²
Casein	270 Cal ²
Nitrogen source "B" ¹	270 Cal ²
Corn gluten	234 Cal

¹ Data from section 4.

² Nitrogen sources of approximately the same quality.

It would appear that as the quality of the nitrogen source increases a corresponding increase occurs in the A.D.E. intake per 100 gm. body weight.

It will be remembered that in the analysis of variance between the A.D.E. intake per 100 gm. body weight of the rats only rations 16, 18 and 19 of the 22 rations containing nitrogen source "A" were involved in the calculations. The average gross nitrogen content of these rations was 1,897 mg. per 100 gm. which is considerably higher than the nitrogen content of the rations based on the other nitrogen sources. However, examination of the data of table 17 indicates the improbability that the level of nitrogen in the rations affected A.D.E. intake. Further evidence that nitrogen level of the ration does not directly influence food, and therefore A.D.E., consumption has previously been presented in sections 3 and 4.

Fisher and Weiss (1956) reported that fibre per se is an important factor, stimulating the feed intake of chicks independently of the energy level of the ration. This may be interpreted to suggest that fibre per se increases the intake of available energy. However, rats on ration 8 of this experiment, the only ration containing no Alphacel, consumed an average of 345 Cal. A.D.E. per 100 gm. body weight compared to a mean of 302 Cal. for rats receiving rations containing Alphacel and based on

the same nitrogen source. These results, though not conclusive, would indicate that fibre tends to depress the energy intake of weanling rats. Fisher and Weiss (1956) derived their energy values from computations involving the Atwater factors for metabolizable energy. In a footnote it was pointed out that Anderson and Hill (1955) have derived a factor for tallow, in chick nutrition, which is considerably lower than that presented by Atwater. If the factor of Anderson and Hill (1955) had been used in the calculations it is doubtful whether fibre as such would have been found to exert a very significant influence on either feed or energy intake. If the factor is more correct than that of Atwater then the variation between the approximately isocaloric rations of Fisher and Weiss (1956) might be of sufficient magnitude as to explain part of the stimulation of feed and energy intake, attributed to fibre, on the basis of a variation in growth rate resulting from differences in the protein: energy ratio. Peterson et al. (1954) reported a stimulation in the feed consumption of chicks when fibre replaced some of the glucose in a ration, but explained this stimulation as being a function of the greater growth promoting effect of the fibre containing rations resulting from the increased ratio of protein to other digestible nutrients. It would appear that to obtain satisfactory information regarding the influence of available energy

and fibre on food consumption, calculations should be based on the results of actual energy determinations and not on calculated figures. The latter are subject to several sources of error such as those involved in mixing the rations and the variation in digestibility between individual animals.

Changes in density were not found to influence the A.D.E. consumption of weanling rats. However, changes in density were closely associated with changes in the A.D.E. content of the food. For this reason a measure of the correlation between the volume of food consumed per 100 gm. body weight and the reciprocal of the A.D.E. per 100 ml. of food yields a higher r value than that between the weight of food consumed per 100 gm. body weight and the reciprocal of the A.D.E. per 100 gm. of food. This supports the findings of section 4.

It has been pointed out that the A.D.E. consumption, per 100 gm. body weight, of weanling rats did not show any significant variation when the A.D.E. per 100 ml. of food varied from 39 to 265 Calories. This indicates that the maximum possible food consumption was not reached even when a ration contained 65% Alphacel.

Nitrogen Retention

The results, relative to the nitrogen retention of rats receiving nitrogen source "A", have demonstrated the

curvilinear relationship between the percentage A.D.N. retained and the A.D.E. per gm. A.D.N. The association between the variables was 91% and a maximum of 86.3% of the A.D.N. was retained when the food contained 265 Cal. per gm. A.D.N. In an earlier experiment (section 3) in which fewer rations were involved but in which the same nitrogen source was employed the corresponding figures were 87%, 89.3% and 277 Cal. A.D.E. per gm. A.D.N. The slight variation between the results of the two experiments may be explained by the considerably greater range of A.D.E. per gm. A.D.N. values, employed in this experiment, which allowed for the formulation of a more reliable curve.

The nitrogen retention data relative to rats receiving rations containing nitrogen sources other than "A" do not allow any definite conclusions to be drawn, because of the small number of rations involved. However, there is an indication that the maximum percentage of A.D.N. retained for each nitrogen source occurred when the A.D.E. per gm. A.D.N. of the food fell within the range of 250 to 300 Calories. Previous experiments have shown maxima to occur in this range (sections 3 and 4). It seems worthwhile to continue studies into the relationship between the A.D.E. per gm. A.D.N. of the food and the A.D.N. retention for, if the maxima always occur in a narrow range for each physiological process e.g. growth,

gestation, lactation etc., the formulation of satisfactory rations would be simpler and would avoid the excessive and uneconomical use of either protein or energy, the two most expensive components of any practical ration.

Summary

Variations in the food consumption of weanling rats, fed rations containing varying nitrogen sources, were largely associated with the A.D.E. content of the rations. A significant difference in the A.D.E. consumption of rats between nitrogen sources was attributed to the quality of the nitrogen sources. The higher the quality of the nitrogen source the greater the A.D.E. consumption. Evidence was presented to suggest that fibre tends to depress A.D.E. consumption. The higher physiological limit of food consumption was not attained even when the ration contained 65% Alphacel.

The nitrogen retention data stressed the importance of the A.D.E.:A.D.N. ratio of the food in controlling the percentage A.D.N. retained. It is possible that the optimum level of A.D.E. per gm. A.D.N. for all nitrogen sources studied (in respect to the growth of weanling rats) was within the range of 250 to 300 Calories.

SECTION 6

SUMMARY AND CONCLUSIONS

The methods and procedures used in the execution of the research reported herein, together with the experimental results obtained, have been presented in some detail; pertinent discussions of probatory findings have also been subsumed. The present section represents a consolidation of the conclusions resulting from the foregoing experiments.

Food Consumption

The food intake of weanling rats is largely controlled by the A.D.E. content of the ration. When measuring the affinity between these two variables it would seem advisable to relate the volume of food consumed to the reciprocal of the A.D.E. content of the diet. It is preferable to use volume rather than weight for measuring food intake as changes in the A.D.E. content of a ration are frequently reflected in an alteration in food density. When measuring food intake, correction should be made for the variance in body weight and/or change in weight between ration groups. Covariance enables such corrections to be made but in short term experiments the simpler method of expressing food intake on a per unit average body

weight basis appears adequate.

Nitrogen per se does not influence either the food or A.D.E. consumption of weanling rats. Even when a nitrogen source composed of the indispensable amino acids and diammonium citrate was employed the level of nitrogen in the diet exerted no detectable influence on food intake. However, A.D.E. intake appeared to vary directly with the quality of the nitrogen source.

Although the evidence was not conclusive it would appear that fibre per se tended to decrease energy consumption. However, there was no apparent variation in the A.D.E. intake of rats receiving rations varying in Alphacel content from 10 to 65 percent.

These findings indicate that in formulating rat rations the various components should be included in a particular ratio to the available energy content of the diet. If the recommended allowance of a nutrient X for the weanling rat is 10 units per 100 gm. body weight per week and if in the same period a similar animal will consume 300 Cal. A.D.E. per 100 gm. body weight then in formulating a ration for such an animal X should be included at a level of one unit per 30 Cal. A.D.E. It is true that the A.D.E. consumption varies with the nature of the nitrogen source, for which an allowance would have to be made, but nevertheless it is felt that such a method of ration formulation would be superior

to the present method by which nutrients are included on a unit weight basis. By using the latter method a deficiency of nutrient X might arise on very high energy rations while a harmful excess might be consumed if the ration was of low available energy content. It is at present unknown whether the feed intake of farm livestock, other than chickens, is controlled by the available energy content of the ration. It is quite possible that such regulation occurs and it is believed that this is a field worthy of further investigation.

Nitrogen Retention

A curvilinear relationship exists between the percentage A.D.N. retained and the A.D.E. per gm. A.D.N. in the food. When measured by maximum nitrogen retention the optimum level of A.D.E. per gm. A.D.N. appears to fall between 250 and 300 Cal. The percentage A.D.N. retained at the maximum varies directly with the quality of the nitrogen source. It will be noted that the value 'percentage A.D.N. retained' differs from the biological value obtained by the Thomas-Mitchell Method only in as much as no corrections are made for metabolic faecal and endogenous urinary nitrogen. It would seem necessary that future biological value determinations should take account of the A.D.E.:A.D.N. ratio.

The level of indigestible material in a ration

influences nitrogen retention in as much as the apparent digestibility of the nitrogen source decreases as the proportion of indigestible material increases.

It is probable that the optimum ratio of A.D.E.: A.D.N. as measured by nitrogen retention will vary between species and stages of development, however, it would seem necessary to further investigate this field if it is intended to make the most efficient use of food nitrogen.

Carcass Composition

There were no significant differences in the protein, fat or moisture content of the carcasses of rats fed rations varying in both A.D.E. and A.D.N. content. This was attributed to the short period (two weeks) for which the rats were subjected to the experimental diets.

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